

Role of the indoor environment in determining the severity of asthma

Ashley Woodcock, Adnan Custovic

North West Lung Centre, Wythenshawe Hospital, Southmoor Road,
Manchester M23 9LT, UK

Introductory articles

Allergen exposure decreases glucocorticoid receptor binding affinity and steroid responsiveness in atopic asthmatics

SR Nimmagadda, SJ Szeffler, JD Spahn, W Surs, DYM Leung

*Allergen exposure can confound the management of asthma. To understand the potential mechanisms by which allergens increase the steroid requirements in atopic asthmatics, we examined the effects of allergens on glucocorticoid receptor (GCR) binding affinity and glucocorticoid (GC) responsiveness of peripheral blood mononuclear cells (PBMC) from atopic asthmatics. A significant reduction ($p < 0.001$) in the GCR binding affinity (K_d) was observed in ragweed-allergic asthmatics during ragweed pollen season compared with PBMC obtained before and after ragweed season. In vitro effects of allergen on PBMC GCR K_d were also examined by incubating PBMC from atopic asthmatics with allergen (ragweed and cat) versus *Candida albicans*. GCR binding affinity was significantly reduced after incubation with ragweed ($p < 0.001$) or cat allergen ($p < 0.001$) compared with baseline or *C. albicans* stimulation. This effect was limited to atopic asthmatics in that in vitro cat allergen incubation for 48 h failed to significantly alter GCR binding affinity in nonasthmatic, atopic individuals. These allergen-induced reductions in GCR binding affinity also rendered the PBMC less sensitive to the inhibitory effects of hydrocortisone and dexamethasone on allergen-induced proliferation ($p < 0.01$). To test the hypothesis that allergen-induced alterations in GCR binding affinity were cytokine-induced, we examined the effects of interleukin-2 (IL-2) and IL-4 neutralization using anticytokine antibodies. Addition of both anti-IL-2 and anti-IL-4 antibodies resulted in a significant ($p < 0.001$) inhibition of allergen-induced alterations in GCR binding affinity. Furthermore incubation with cat allergen induced significantly higher concentrations of IL-2 ($p = 0.03$) and IL-4 ($p = 0.02$) by PBMC from atopic as compared with nonatopic subjects. Our current observations suggest that allergen exposure may contribute to poor asthma control by reducing GCR binding affinity in mononuclear cells. This appears to be mediated through IL-2 and IL-4. These findings may have important implications for novel approaches to the treatment of poorly controlled asthma. (Am J Respir Crit Care Med 1997;155:87–93)*

Allergen reduction measures in houses of allergic asthmatic patients: effects of air-cleaners and allergen-impermeable mattress covers

S van der Heide, HF Kauffman, AEJ Dubois, JGR de Monchy

Recommendations for allergen avoidance or allergen reduction measures play an important part in the treatment of allergic asthmatic patients. The purpose of this study was to test recently developed air-cleaners with respect to their capacity to capture airborne allergen particles and to improve clinical parameters of asthmatic patients sensitized to aeroallergens. Forty five allergic asthmatic patients were studied in a double-blind procedure for 6 months. The patients were divided into three groups of 15 patients. In Group 1, the intervention consisted of the application of active air-cleaners in living-rooms and bedrooms. In Group 2, placebo air-cleaners were used in combination with allergen-impermeable mattress covers. In Group 3, the same intervention was performed as in Group 2 but with active air-cleaners. Allergen levels in mattress and floor dust were measured before, and 3 and 6 months after the interventions. After 6 months, the air-cleaners were dismantled and the filters were analysed for the amount of dust collected and allergen content. Immunological and lung function parameters were

measured before, and 3 and 6 months after the interventions. Considerable amounts of airborne dust and allergic particles were captured in the filters of the air-cleaners. Up to the 18.9 g of dust, 4,513 ng of house dust mite allergen, Der p 1, and 50,000 mU of cat allergen, Fel d 1 (in houses with cats) were collected by air-cleaners in living-rooms. Only in Group 3 (in which both active air-cleaners and mattress covers were used) was a small (less than 1 doubling dose) but statistically significant improvement of provocative concentration of histamine causing a 20% fall in forced expiratory volume in one second (PC_{20}) observed (from 5.96 to 9.02 $mg \cdot ml^{-1}$). The amount of dust and house dust mite allergen collected in the filters was significantly correlated with an improvement of peak flow variation. In combination with other allergen avoidance measures, the examined air-cleaners can contribute to diminished allergen exposure and improvement of airway hyperresponsiveness in asthmatic patients. (*Eur Respir J* 1997; 10:1217–1223)

Asthma doctors tend to fall within a spectrum of two extremes, either enthusiasts for allergy or for pharmacotherapy. The former see asthma as an extrinsic problem which can be controlled by the strict avoidance of offending environmental insults or by immunotherapy. In their support, there are differences in the prevalence of asthma between the same genetic stock in different environments, and also convincing evidence for increasing asthma prevalence with time. And the environment is changing; in addition to changes in ventilation, furnishings and house construction, in the UK there are now 15 million dogs and cats sharing our houses (more pets than children). On the other hand, the pharmacologically orientated physician sees asthma as an intrinsic airways disease involving whichever inflammatory cell and mediator is fashionable this year. In the 1970s this was cyclic AMP, in the 1980s PAF, and in the 1990s various cytokines and T lymphocytes. Cynics compare asthma to an inflammatory goulash soup and argue that ever more detailed pea splitting will not help patients. Perhaps we should try to work out what is heating up the soup in the first place—namely, the environment. Whilst this is said tongue-in-cheek, there is more than a little truth in it.

However, we still need drugs to control established asthma. Glucocorticosteroids are the cornerstone of anti-inflammatory treatment of asthma. The therapeutic effect of steroids varies between patients with chronic asthma and is paralleled with a varying degree of glucocorticoreceptor (GCR) binding affinity in peripheral blood mononuclear cells (PBMCs).¹ Some asthmatic patients are relatively resistant to therapy with inhaled steroids. In many patients this may be due to poor compliance, but for other subjects this is a true drug resistance which may in theory be inherited or acquired. It has been known for some time that glucocorticoid binding affinity is reduced in patients with steroid resistant asthma.² This abnormality normalises after 48 hours in culture, but can be restored by co-incubation in a medium containing IL-4 and IL-2.

In addition, the airways of steroid resistant asthmatics are known to have higher IL-2 and IL-4 cytokine gene expression.² This implies that the binding of steroids to PBMCs is influenced by cytokines. Identifying factors which are associated with steroid resistance would have important diagnostic and therapeutic implications.

Allergen exposure and steroid responsiveness

The first introductory paper by Nimmagadda *et al* starts to bridge the gap between pharmacologists and allergists, and provides evidence that allergen exposure may confound the pharmacological management of the dis-

ease.³ This group investigated the effect of allergen exposure on GCR binding affinity and responsiveness to glucocorticoids of PBMCs in atopic asthmatics both in vivo and in vitro. The effect of in vivo exposure was tested in patients allergic to ragweed before, during and after the ragweed pollen season. A significant reduction in GCR binding affinity was observed during the pollen season compared with pre-season and post-season measurements (fig 1). It is of interest that there was no difference between the patients on inhaled steroids and those on β_2 agonists only.

The effect of in vitro allergen exposure was also investigated by incubating PBMCs from atopic asthmatics with either the relevant allergen to which they were sensitised or *Candida albicans* (as a control). PBMCs of ragweed allergic asthmatics obtained outside the pollen season and of cat allergic patients not exposed to cats had significantly reduced GCR binding affinity after incubation with ragweed and cat allergen, respectively, compared with both baseline and *Candida albicans* stimulation. This confirms that the observed effect is allergen specific and is restricted to atopic asthmatic patients as no similar effect was found in atopic non-asthmatic individuals or non-atopic subjects. Furthermore, the allergen induced reduction in GCR binding affinity of PBMCs from atopic asthmatic subjects made the lymphocytes significantly less responsive to the inhibitory effect of hydrocortisone.

Cat allergen may induce IL-2 and IL-4 production from PBMCs of sensitised individuals. The incubation

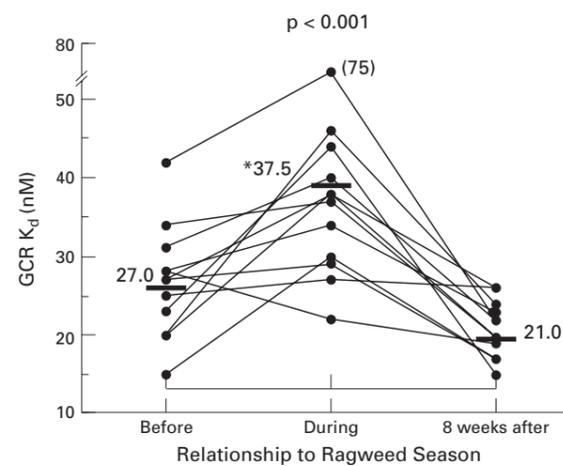


Figure 1 Effect of exposure to ragweed pollen on GCR binding affinity in patients allergic to ragweed. Reproduced with permission from Nimmagadda *et al*.³

of PBMCs from atopic asthmatics with cat allergen and either anti-IL2 or anti-IL4 antibodies inhibited the previously observed reduction in GCR binding affinity, suggesting that IL-2 and IL-4 may play an important role in altered steroid responsiveness, and that the effect of cat allergen is mediated via these cytokines. The finding that allergen exposure reduces GCR binding affinity in atopic asthmatics both in vivo and in vitro with the resulting functional alteration in cellular response to glucocorticoids supports the view that high allergen exposure in sensitised individuals may contribute to poor asthma control and maintenance of the inflammatory process in the airways, in spite of treatment with inhaled steroids.

Showing an effect of environment on inflammation or drug responsiveness is important, but what happens when patients try to reduce allergen exposure? In the past, many studies have looked at allergen exposure and allergen sensitisation separately as risk factors for the development and severity of asthma. Since house dust mites are the commonest source of allergens in temperate climates, we will look at the relationship between exposure to mites, atopic sensitisation, and asthma in more detail.

Allergens and asthma

The relationship between mites and asthma is complex and can be investigated with respect to: (1) exposure to mites and allergic sensitisation; (2) allergic sensitisation and asthma; (3) exposure to mites and asthma development; and (4) exposure to mites and asthma symptoms.

EXPOSURE TO MITE ALLERGEN AS A RISK FACTOR FOR SENSITISATION

In a German Multicentre Atopy Study 1314 newborn babies were selected for a prospective study of the influence of exposure to indoor allergens on atopic sensitisation (499 at high risk; remaining 815 chosen at random).⁴ Both dust samples (for determination of allergen exposure) and blood samples (for determination of sensitisation) were available for 764 children. Children sensitised to dust mite had a significantly higher level of mite allergen in their homes than those in the non-sensitised group (868 ng/g vs 210 ng/g). In a recent study in Linköping (Sweden) Munir *et al* followed a group of 86 children at high risk from birth until five years of age.⁵ Eleven children developed sensitisation to indoor allergens, all 11 to cats, three to dogs, and one to dust mites. The finding that only one of 86 children in this study was sensitised to mites during the first five years of life, despite a strong family history of allergic disease, may relate to the very low exposure to mite allergens (the highest level recorded was only 3.5 µg/g). In a recent cross sectional study from Norway, sensitisation to mites correlated strongly with levels of mite allergen in mattresses (corresponding to odds ratio (OR) of 16).⁶ Warner *et al* reported that in three areas of Sweden the prevalence of sensitivity to dust mites and the level of mite specific IgE were both significantly associated with the concentration of mite allergen in homes.⁷

Early infancy has been identified as a critical period for primary sensitisation. Evidence to support this view comes from studies relating atopy to month of birth. Exposure to pets in early infancy is associated with specific IgE sensitisation and allergic disease later in childhood. Exposure to Der p 1 at a level of >2 µg/g dust during infancy is associated with increased prevalence of

positive skin tests and increased concentrations of IgE specific to dust mite by the age of five in children of atopic parents.⁸

SENSITISATION TO MITE ALLERGEN AS A RISK FACTOR FOR ASTHMA

On the east coast of the USA sensitisation to mite allergens is the single strongest risk factor for asthma among middle school children.⁹ Wickman *et al* reported that even in an area with low mite allergen exposure (Sweden) there was a close association between mite sensitisation and asthma (OR 4.9).¹⁰ Leung *et al* have recently reported that sensitisation to allergens was a significant risk factor for asthma in Chinese populations living in three different areas of south-east Asia, with adjusted ORs for mite sensitisation being between 1.9 and 5.8.¹¹ In a group of 68 children of atopic parents prospectively followed from birth in Poole, Dorset, sensitisation to mites at the age of 10 years was associated with an OR of 19.7 for having asthma.¹²

EXPOSURE TO MITE ALLERGEN AS A RISK FACTOR FOR ASTHMA

A recent study of children from six regions of New South Wales, Australia with a range of levels of mite allergens has provided further strong evidence on the role of exposure to mite allergens in childhood asthma.¹³ Children who were skin test positive to mites in each of the areas were at significant risk for having asthma, with the risk of mite sensitised children having asthma doubling for every doubling of the current level of exposure to mite allergens. In a previously mentioned study from Poole, exposure to Der p 1 at a level of >10 µg/g measured in infancy was associated with a 4.8 fold relative risk of developing atopic asthma at the age of 11.¹²

EXPOSURE TO MITE ALLERGEN AND ASTHMA SYMPTOMS

It is difficult to demonstrate the dose-response relationship between exposure to indoor allergen and asthma severity. Many asthmatic patients are sensitised and exposed to more than one allergen, and the relative contribution of each to the airway inflammation may be difficult to elucidate. "Trigger factors" such as viruses, air pollution, exercise, and emotion, may make asthma severity worse. In addition, some sensitised patients react to a very low dose of allergen, whilst in others the level required to cause symptoms is considerably higher. Nevertheless, when Peat *et al* compared children living in areas with different levels of exposure to mites, they found a similar prevalence of mite sensitivity in both regions, but more severe bronchial hyper-reactivity in sensitised children in the area with the highest mite levels.¹⁴ We have found a modest but significant correlation between the concentration of Der p 1 in the bed and objective indices of asthma severity (non-specific bronchial hyperreactivity, FEV₁ and PEF variability) in mite sensitised adult asthmatics, but not for patients with negative skin prick tests to mites.¹⁵

We have recently shown that exhaled nitric oxide (NO), a marker of bronchial inflammation, is increased in asthmatics who are both sensitised and exposed to the relevant allergen compared with those who are sensitised but not exposed.¹⁶ In addition, patients with more severe asthma, and especially those with brittle asthma, are both sensitised and exposed to high levels of pet allergens.¹⁷

On the basis of current evidence it seems likely that sensitised patients exposed to high levels of allergens will usually have more severe disease than those exposed to low levels of allergens and that they should benefit from the effective environmental control.

Allergen avoidance

Effectiveness of allergen avoidance in the treatment of asthma was first suggested in studies in which patients were removed from their homes containing high levels of mite allergen into environments where allergen levels were low. In Europe the mite population density is very low at high altitudes (>1500 m) where humidity is insufficient to support mite survival. A number of studies conducted at high altitude sanatoria (e.g. Davos, Misurina) have demonstrated an improvement in non-specific bronchial hyperresponsiveness and symptoms with re-exposure resulting in a rapid relapse.¹⁸ The high altitude studies were not controlled and it is possible that other domestic factors such as pets, environmental tobacco smoke, or indoor pollution contributed to the observed improvement in asthma control. The lesson from high altitude studies is that it is essential to achieve and maintain a major reduction in allergen levels, and that even with such a reduction in exposure it may take many months for the improvement in asthma control to become fully apparent.

The important practical question is whether allergen levels in homes can be reduced sufficiently to improve asthma control in sensitised patients. We have recently reviewed the practical measures for allergen avoidance.¹⁸ There are conflicting data on the effectiveness of allergen avoidance carried out in houses, primarily because the majority of studies have been small, poorly controlled, and have often used measures that do not reduce mite allergen exposure. Consequently, many fail to show clinical benefits and, as a result of low power, it is not clear which subgroups of patients will benefit most from the reduction in allergen exposure. Double blind, placebo controlled trials of allergen avoidance are difficult to conduct. Almost every controlled study has observed a significant reduction in mite allergen levels and sometimes improved clinical symptoms in both the control group as well as the active group.¹⁸

The second introductory paper by van der Heide *et al* highlights the difficulties faced by investigators when conducting studies of allergen avoidance.¹⁹ In this trial a relatively small number (45) of house dust mite sensitive adults with mild asthma were randomised into one of the three groups: group 1, active air cleaners in living rooms and bedrooms; group 2, placebo air cleaners and mattress and pillow covers; group 3, active air cleaners and mattress and pillow covers. Unfortunately, in addition to house dust mite, most of the patients were also sensitised to other inhalant allergens (22 to pollens and cats; four of the cat allergic patients lived in a home with a cat). Mattress covers were once again shown to be very effective in reducing the amount of mite allergen Der p 1. Unexpectedly, the placebo air cleaners (essential to prevent unblinding of patients and investigators) also captured dust and allergens on the coarse filter. Using changes in the airway hyperresponsiveness as a primary outcome measure, in the multiple regression analysis the authors have shown that the greatest improvement in PC₂₀ histamine was found in patients who had textile floor covering in the living room, possessed domestic animals, and experienced the largest decrease in the concentration of mite allergen Der p 1 in mattress dust.

This study raises two important questions, unfortunately without providing the answer to them: (1) what are the most appropriate avoidance measures for individual patients with asthma and (2) who are the patients who will benefit most from allergen avoidance?

WHAT ARE THE MOST APPROPRIATE AVOIDANCE MEASURES FOR INDIVIDUAL PATIENTS WITH ASTHMA?

Mite allergens can be detected in the air in significant amounts only after vigorous disturbance²⁰ and are predominantly contained within relatively large particles (>10 µm diameter). We deduce that most mite allergen exposure is from bedding, and that bed covering ought to be an effective control measure. In contrast, cat and dog allergens are readily measured in the air and ~25% of airborne Fel d 1 and Can f 1 is associated with small particles (<5 µm diameter).^{21,22} This would imply that air filtration units have little place in mite avoidance but may be useful in removing cat and dog allergens from the air (this may be the reason why pet owners in van der Heide's study improved more than non-pet owners). Maybe the best approach in the future would be to think about integrated measures for avoiding all indoor allergens to which patients are sensitised rather than concentrating on a single allergen.

WHO ARE THE PATIENTS WHO WILL BENEFIT MOST FROM ALLERGEN AVOIDANCE?

It would appear that the patients benefiting most from allergen avoidance were those exposed to the highest levels of allergens before intervention (it is likely that these were the ones with the largest reduction in mite allergen levels; in addition, it is possible that the presence of carpets in living rooms could have been a marker of high allergen exposure as carpeted homes contain much larger quantities of mite allergens than those without carpets). This underlines the importance of individual measurements of exposure in sensitised asthmatic patients and stresses the need for a simple, cheap and effective way of assessing individual exposure. Novel semi-quantitative monoclonal antibody based immunodot strip techniques for the detection of house dust mite, cat, and cockroach allergen are currently being developed and may provide us with a tool for assessing individual exposure in the near future.

Finally, is it mild asthmatics or those with severe disease who will benefit most from the effective avoidance of relevant allergens? The study by Nimmagadda *et al* suggests that patients with poorly controlled asthma and steroid resistant asthma are the ones who would benefit most from allergen reduction (but only to the allergens to which patients are both sensitised and exposed, both in terms of decreasing the airway inflammation with consequent improvement in non-specific bronchial hyperresponsiveness and symptoms, and in improving their response to treatment with glucocorticosteroids). One of the reasons behind the spectacular results obtained in high altitude sanatoria could be that it is usually more severe patients who are referred to these centres rather than relatively milder asthmatics used in trials of allergen avoidance at home (e.g. the PC₂₀ histamine in the previously reviewed study by van der Heide was ~7 mg/ml).¹⁹

Conclusions: allergen avoidance in tertiary prevention of asthma

The cost of asthma care is increasing. It seems likely

LEARNING POINTS

- * Allergen exposure has been shown to reduce glucocorticoid receptor binding affinity and glucocorticoid responsiveness of peripheral blood mononuclear cells in atopic asthmatic subjects.
- * This link may be important in explaining reduced efficacy of steroid anti-inflammatory therapy in some asthmatic subjects.
- * This study is important as it demonstrates a potential link between environmental allergen exposure and pharmacotherapy in asthma.
- * The relation between allergen exposure and asthma is complex and multi-faceted.
- * Studies of house dust mite avoidance on incidence and severity of asthma are methodologically difficult and have produced conflicting data.
- * Mite avoidance may become an important component in the management strategy of asthma following further well designed studies.

that an integrated approach which combines anti-inflammatory treatment (usually with an adequate dose of inhaled steroid) with environmental control of the relevant allergens (those to which the patient is sensitised) will be the most successful. Since mite sensitisation and exposure are most relevant in temperate climates, an effective, simple and widely applicable environmental intervention could have major benefits. While the overall effect of an intervention may be small at an individual level, in population terms this could still translate into clinically important and worthwhile health benefits. A randomised, blinded and controlled trial of house dust mite avoidance for one year is about to commence in the UK with 2000 enrolled asthmatic patients. Only studies such as this with adequate power will show which patients (and in which domestic environments) will benefit from the intervention. The results of such trials, along with long term studies of primary allergen avoidance, are awaited with interest.

- 1 Spahn JD, Leung DYM, Surs W, *et al.* Reduced glucocorticoid binding affinity in asthma is related to ongoing allergic inflammation. *Am J Respir Crit Care Med* 1995;151:1709-14.
- 2 Sher ER, Leung DYM, Surs W, *et al.* Steroid resistant asthma: cellular mechanisms contributing to inadequate response to glucocorticoid therapy. *J Clin Invest* 1994;93:33-9.
- 3 Nimmagadda SR, Szeffler SJ, Spahn JD, *et al.* Allergen exposure decreases glucocorticoid receptor binding affinity and steroid responsiveness in atopic asthmatics. *Am J Respir Crit Care Med* 1997;155:87-93.
- 4 Wahn U, Lau S, Bergmann R, *et al.* Indoor allergen exposure is a risk factor for sensitisation during the first three years of life. *J Allergy Clin Immunol* 1997;99:763-9.
- 5 Munir AKM, Kjellman NIM, Bjorksten B. Exposure to indoor allergens in early infancy and sensitization. *J Allergy Clin Immunol* 1997;100:177-81.
- 6 Dotterud LK, Van TD, Kvammen B, *et al.* Allergen content in dust from homes and schools in northern Norway in relation to sensitisation

- and allergy symptoms in schoolchildren. *Clin Exp Allergy* 1997;27:252-61.
- 7 Warner AM, Bjorksten B, Munir AKM, *et al.* Childhood asthma and exposure to indoor allergens: low mite levels are associated with sensitivity. *Pediatr Allergy Immunol* 1996;7:61-7.
- 8 Rowntree S, Cogswell JJ, Platts-Mills TAE, *et al.* Development of IgE and IgG antibodies to food and inhalant allergens in children at risk of atopic disease. *Arch Dis Child* 1985;60:727-35.
- 9 Squillace SP, Sporik RB, Rakes G, *et al.* Sensitisation to dust mites as a dominant risk factor for asthma among adolescents living in Central Virginia. Multiple regression analysis of a population-based study. *Am J Respir Crit Care Med* 1997;156:1760-4.
- 10 Wickmann M, Nordvall SL, Pershagen G, *et al.* Sensitisation to domestic mites in a cold temperate region. *Am Rev Respir Dis* 1993;148:58-62.
- 11 Leung R, Ho P, Lam CWK, *et al.* Sensitisation to inhaled allergens as a risk factor for asthma and allergic disease in Chinese population. *J Allergy Clin Immunol* 1997;99:594-9.
- 12 Sporik R, Holgate S, Platts-Mills TAE, *et al.* Exposure to house dust mite allergen (Der p1) and the development of asthma in childhood. *N Engl J Med* 1990;323:502-7.
- 13 Peat JK, Tovey ER, Toelle BG, *et al.* House dust mite allergens: a major risk factor for childhood asthma in Australia. *Am J Respir Crit Care Med* 1996;152:144-6.
- 14 Peat JK, Tovey E, Gray EJ, *et al.* Asthma severity and morbidity in a population sample of Sydney schoolchildren: Part II. Importance of house dust mite allergens. *Aust NZ J Med* 1994;24:270-6.
- 15 Custovic A, Taggart SCO, Francis HC, *et al.* Exposure to house dust mite allergens and the clinical activity of asthma. *J Allergy Clin Immunol* 1996;98:64-72.
- 16 Simpson A, Custovic A, Adisesh A, *et al.* Exhaled nitric oxide (eNO) in relation to sensitisation and exposure to indoor allergens in asthmatics. *J Allergy Clin Immunol* 1998;101:S235.
- 17 Fletcher TJ, Tunnicliffe W, Hammond K, *et al.* Domestic allergen exposure in brittle asthma. *Am J Respir Crit Care Med* 1996;153:A514.
- 18 Custovic A, Simpson A, Chapman MD, *et al.* Allergen avoidance in the treatment of asthma and atopic disorders. *Thorax* 1998;53:63-72.
- 19 van der Heide S, Kauffman HF, Dubois AEJ, *et al.* Allergen reduction measures in houses of allergic asthmatic patients: effects of air cleaners and allergen-impermeable mattress covers. *Eur Respir J* 1997;10:1217-23.
- 20 Custovic A, Taggart SCO, Niven RMCL, *et al.* Monitoring exposure to house dust mite allergens. *J Allergy Clin Immunol* 1995;96:134-5.
- 21 Custovic A, Green R, Fletcher A, *et al.* Aerodynamic properties of the major dog allergen, Can f 1: distribution in homes, concentration and particle size of allergen in the air. *Am J Respir Crit Care Med* 1997;155:94-8.
- 22 Custovic A, Smith A, Pahdi H, *et al.* Distribution, aerodynamic characteristics and removal of the major cat allergen Fel d 1 in British homes. *Thorax* 1998;53:33-8.